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(71) Applicant *(for all designated States except US)*: THE REGENTS OF THE UNIVERSITY OF MICHIGAN [US/US]; Technology Management Office, Wolverine Tower, Room 2071, 3003 South State Street, Ann Arbor, MI 48109-1280 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MEYERHOFF, Mark, E. [—/US]; 1312 Shevchenko, Ann Arbor, MI 48103 (US). BATCHELOR, Melissa, M. [—/US]; University of Michigan, Department of Chemistry, 3316 Chemistry, Ann Arbor, MI 48105 (US). OH, Bong, Kyun [—/US]; University of Michigan, Department of Chemistry, 3316 Chemistry, Ann Arbor, MI 48105 (US).

(74) Agent: ROHM, Benita, J.; Rohm & Monsanto, PLC, 660 Woodward Avenue, Detroit, MI 48226 (US).

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(54) Title: BIOCATALYTIC AND BIOMIMETIC GENERATION OF NITRIC OXIDE *IN SITU* AT SUBSTRATE/BLOOD INTERFACES

(57) Abstract: Biocompatible materials that have the ability to release nitric oxide (NO) *in situ* at the surface-blood interface when in contact with blood. The materials which may be polymers (e.g., polyurethane, poly(vinyl chloride), silicone rubbers), metals, such as stainless steel, carbon, and the like are provided with biocatalysts or biomimetic catalysts on their surface that have nitrite, nitrate and/or nitrosothiol-reducing capability that. Illustratively, the catalysts are adsorbed or immobilized at the surface of the material. The catalysts can act on endogenous nitrite/nitrate or nitrosothiols within the blood creating a local increase in the NO levels at the surface of the material. An illustrative enzymatic biocatalyst is mammalian xanthine oxidase. In another illustrative embodiment, a biomimetic catalyst is a copper (Cu(II)-ligands complex, e.g., dibenzo[e.k]-2,3,8,9-tetraphenyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene. In some cases, lipophilic salts of nitrite/nitrate (e.g., tridodecylmethylammonium nitrite ($TDMA^+NO_2^-/NO_3^-$)) or certain salts of nitrosothiols can be doped within a polymer material, or an underlying polymeric film, to create a reservoir of nitrite or nitrosothiol that continuously leaks into the immobilized catalytic layer. Adequate levels of endogenous reducing equivalents are present within blood to provide catalytically-generated surface levels of NO that are above the threshold reportedly required to prevent platelet adhesion or activation.

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Biocatalytic and Biomimetic Generation of Nitric Oxide *in situ* at Substrate/Blood Interfaces

Government Rights

This invention was made under contract awarded by the National Institutes of Health, Contract Number F-002881. The government has certain rights in the invention.

Relationship to Other Application(s)

This application is a continuation-in-part of U.S. Serial No. 60/262,014 filed on January 16, 2001, and claims the benefit thereof.

Background of the Invention

FIELD OF THE INVENTION

This invention relates generally to biocompatible materials, such as polymers or metals, and more particularly, to biocompatible materials having blood interface surfaces that are capable of biocatalytic or biomimetic generation of nitric oxide *in situ* when contacted with endogenous nitrite/nitrate or nitrosothiols in blood.

DESCRIPTION OF THE RELATED ART

Although medical devices such as extracorporeal circuits and hemodialysis tubes are widely used in clinical settings, the polymers typically used to fabricate such devices (PVC, polyurethane, silicone rubber, *etc.*) are still subject to platelet aggregation and adhesion onto the surface of these materials. Thus, patients are often given anti-clotting agents (*i.e.*, heparin) in order to reduce thrombosis on the surface of these devices. Similarly, implanted devices made of stainless steel or other alloys, or even carbon, can cause thrombus formation when in direct contact with blood. There is, therefore, a need for materials that more closely simulate the antithrombogenic properties of the endothelial cells that line blood vessels in order to obviate the need to administer anticoagulants.

Nitric oxide (NO) is an important intra- and intercellular messenger molecule that plays an important physiological role in platelet anti-activation, vascular relaxation, neurotransmission, and immune response. It has been proposed that

synthetic materials that release low levels of NO would, therefore, more closely simulate the natural activity of endothelial cells, and therefore, would have improved biocompatibility.

Several classes of NO-releasing materials are currently under investigation worldwide. These include NO donors (*i.e.*, diazeniumdiolates, nitrosothiols) are relatively complicated to synthesize and require stringent storage conditions. Thus, there is a need for improved materials that are easier to fabricate and store.

Currently, NO generation is determined by water uptake (such as in the case of diazeniumdiolates) or the intensity of light (as with iron nitrosyls). However, blood already contains a host of species that are derived from, or are physiologically-generated *in vivo* that can be reduced to NO. These species include, nitrites, nitrates, and a host of nitrosothiols (*e.g.*, nitrosoglutathione, nitroso albumin, *etc.*). This raises the possibility of recycling these species back to nitric oxide. There is, therefore, a need for materials that can reduce these species to nitric oxide locally at the substrate/blood interface.

It is an object of this invention to provide improved materials for biomedical applications that are capable of releasing NO from blood-contacting surfaces materials, so as to prevent platelet activation and adhesion onto these surfaces, thereby lowering thrombus formation and other complications associated with interactions between blood and foreign materials.

It is a further object of this invention to provide improved materials for biomedical applications that are relatively inexpensive to manufacture and that have improved biocompatibility.

It is still a further object of this invention to provide materials for biomedical applications that are capable of releasing NO from blood-contacting surfaces materials in response to nitrites/nitrates and nitrosothiols in the blood.

Summary of the Invention

The foregoing and other objects are achieved by this invention which provides a novel approach for enhancing the biocompatibility of materials of the type suitable

for implantation in a human or animal body and/or for prolonged contact with the body or blood. In accordance with a broad aspect of the invention, materials have been developed to have a catalytic surface that is capable of generating, at the catalytic surface/blood interface, physiologically significant amounts of NO when in contact with blood. A catalytic agent, having nitrite reductase and/or nitrite reductase-like activity, or a nitrosothiol reductase activity, is immobilized, adsorbed, adhered, or otherwise made available at a surface of the material.

In some embodiments, the catalytic agents are biocatalysts, such as enzymes, having nitrite reductase and/or nitrite reductase-like activity, or a nitrosothiol reductase activity. Illustratively,

nitrite reductases, nitrate reductases, enzymes having nitrosothiol reducing ability, and xanthine oxidase, or combinations thereof. Due to the ease of procuring xanthine oxidase commercially(*e.g.*, Sigma, St. Louis, MO), xanthine oxidase is a preferred embodiment. Other potentially useful immobilized biocatalysts would include nitrite reductases and nitrate reductases from plants or bacteria.

In other embodiments, the catalytic agent is a biomimetic catalytic agent. As used herein the term "biomimetic catalytic agent" refers to a species possessing nitrite reductase-like activity, or the ability to reduce nitrosothiols which converts endogenous or exogenous nitrite/nitrate or nitrosothiols to NO when in contact with blood.

Illustratively, the biomimetic catalytic agent is a metal ion ligand complex wherein the metal ion is capable of reducing one or more of nitrate, nitrate, nitrosothiols, and other blood species to nitric oxide. In particularly preferred embodiments, the metal ion ligand complex is a Cu(II) complex. Neutral carrier type ligands that have high metal binding affinity, particularly for copper, and, preferably, planar square-type geometry that provides a minimum amount of steric hindarance to the approach of the electron source (*e.g.*, ascorbate or NADH) to the center metal of the complex so that the copper ion can easily be reduced from Cu(II) to Cu(I), are suitable for the practice of the invention. Examples include, without limitation, nitrogen or sulfur donating compounds, such as N_x-donor macrocyclic ligands (x=2,

4, 5, 6, 8) such as cyclen, cyclam and their derivatives, and crown ethers and S_x-donor macrocyle-type ligands (x=2, 4, 5, 6, 8).

In specific illustrative embodiments, the biomimetic catalyst is a Cu(II) metal ion ligand complex is selected from the group consisting of dibenzo[e,k]-2,3,8,9-tetraphenyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene; dibenzo[e,k]-2,3,8,9-tetramethyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene; and dibenzo[e,k]-2,3,8,9-tetraethyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene.

As used herein, the term "material," when referring to the material that is provided with the catalytic surface, may be any material, and preferably a material of a type that suitable for contact with the body and/or body fluids, particularly blood, of a living being, e.g., is physiologically acceptable, and non-toxic. In some embodiments, the material should be suitable for long-term contact, or in-dwelling uses. Broadly, such materials encompass polymers, metals and alloys, and carbon (graphite).

Many polymeric materials are suitable for the practice of the invention, and the following illustrative list of polymers that have been used for biomedical applications, is illustrative, and not intended to be limiting in any manner. Examples include synthetic polymers such as polyurethane, polydimethylsiloxane, ethylene vinyl acetate, nylons, polyacrylic, polymethyl methacrylate, polyamide, polycarbonate, polyester, polyethylene, polypropylene, polystyrene,polyvinyl chloride, polytetrafluoroethylene, and cellulose acetate.

In specific preferred embodiments, a material in accordance with the invention comprises a hydrophobic polymer substrate, such as poly(vinyl chloride), polyurethane, and silicone rubber, and a layer of a catalytic agent having nitrite reductase and/or nitrite reductase-like activity, or a nitrosothiol reductase activity attached to a surface of the hydrophobic polymer substrate. The attachment may be by adsorption, covalent bonding, and the like. The polymer substrate may, in some embodiments, include lipophilic salts of nitrite/nitrate or nitrosothiols within its matrix to create a

reservoir of nitrite/nitrate or nitrosothiol that can continuously leak to the catalytic surface.

In embodiments where the "material" is a polymer, the NO-releasing polymer can be formed, cast, or otherwise shaped to comprise a monolithic device, such as implantable device such as drug depot or in-dwelling devices, such as catheters, or extracorporeal tubing sets (including kidney dialysis or open-heart surgery heart-lung machines). The polymer can also be applied as a film on another substrate that may be a polymer, or another surface, such as the surface of a metal device.

Suitable metals include, but are not limited to, stainless steel, nickel, titanium, aluminum, copper, gold, silver, platinum and combinations thereof. The metal material may comprise a medical devices, and the following types of devices, provided with a catalytic agent in accordance with the principles of the invention, are meant to be illustrative, but not limiting, examples: arterial stents, guide wires, catheters, bone anchors and screws, protective platings, hip and joint implants, spine appliances, electrical leads, biosensors and probes.

In specific preferred embodiment, the material may comprise a metal substrate that has a biomimetic catalytic agent covalently attached to its surface. As stated above, the biomimetic catalytic agent is a metal ion ligand complex which is capable of reducing one or more of nitrate, nitrate, nitrosothiols, and other blood species to nitric oxide. In particularly preferred embodiments, the biomimetic catalytic agent is a Cu(II) metal ion ligand complex. Attachment of the metal ion ligand to the metal surface may be accomplished by means known to a person of ordinary skill in the art. One such technique involves silanizing the surface of the metal to provide reactive sites to bind the ligand.

In certain preferred embodiments, an exogenous source of nitrite/nitrate or nitrosothiols is provided in the polymer matrix to create a reservoir of nitrite/nitrate or nitrosothiol that can continuously leak to the catalytic surface of the material. In these embodiments, the exogenous source, illustratively, lipophilic salts of nitrite/nitrate or nitrosothiols are dispersed within a polymer matrix material. In some

embodiments, the polymeric material containing the exogenous source of nitrite/nitrate or nitrosothiol is applied to a catalytic surface as a coating. Illustrative source of nitrite/nitrate or nitrosothiol, include, without limitation, quaternary ammonium salts, such as tridodecylmethylammonium nitrite ($TDMA^+$ NO_2^-/NO_3^-); trimethyl phenyl ammonium; dimethyl dioctadecyl ammonium; *etc.* In addition to quaternary ammonium salts, quaternary phosphonium salts or quaternary arsonium salts may be used in the practice of the invention.

Methods of making the invention include swelling a polymer, such as polyvinyl chloride (PVC) or silicone, in the presence of an organic solvent containing an appropriate nitrite/nitrate salt to form a nitrite/nitrate salt-containing polymer. The nitite/nitrate salt-containing polymer is then coated with a layer of immobilized enzyme, illustratively a nitrite reductase enzyme, such as xanthine oxidase. Many techniques are available for immobilizing enzymes. For example, see, Hasselberger, "Uses of Enzymes and Immobilized Enzymes, Nellson-Hall," Chicago (1978) or Guilbault, "Analytical Uses of Immobilized Enzymes," Marcel Dekker, New York (1984).

In a other method embodiments, the biomimetic generation of NO can be further achieved by immobilizing metal-ion ligand complexes, on the surface of the material, or by dispersing these ligands within the material, which may be a polymer. In some embodiments, additional lipophilic nitrite/nitrate salts, or nitrosothiols, are added to an underlying polymer matrix material or provided as a coating on the material, or as an additional layer.

Brief Description of the Drawing

Comprehension of the invention is facilitated by reading the following detailed description, in conjunction with the annexed drawing, in which:

Fig. 1 is a schematic of illustration of NO generation in solution via nitrite reductase activity from the catalytic surface of in a polymer loaded with nitrite salt;

Fig. 2 is a graphical representation of the NO-release profile from nitrite ion-pair doped polymer films having immobilized XO on the surface in the presence of sheep blood;

Fig. 3 is a schematic representation of NO generation from a polymer matrix that has been loaded with a nitrate salt and a Cu(II) ligand complex in accordance with the invention;

Fig. 4 is a schematic representation of a material, in accordance with the invention, wherein a Cu(II) ligand complex is covalently tethered to the surface;

Fig. 5 is a is a graphical representation of the surface generation of NO from a Cu(II) ligand complex-containing polymer film in a bulk solution containing nitrite and ascorbate;

Fig. 6 shows three examples of illustrative metal ligand complexes; and

Fig. 7 is a graphical representation of NO generation from a nitrite ion pair/Cu(II) complex, specifically the complex designated L2 on Fig. 6.

Detailed Description

In one method embodiment for making an improved NO-releasing polymer, the desired polymer may be swelled in an organic solution containing the lipophilic nitrite/nitrate salt. In other embodiments, the salt can be added during the processing stage when the desired end product is molded or cast from the native polymer material. In still other embodiments, the surface of the polymer material that will be exposed to blood, for example, the outside surface of a catheter or the inner surface of tubing of the type used in extracorporeal circuits, or the surface of metal stents, may be coated, either by dip-coating or by another method with a biocatalyst (enzyme) or a biomimetic catalyst capable of reducing nitrate to NO or nitrite to NO, or nitrosothiols to NO. The biocatalysts or biomimetic catalysts can also be covalently tethered to the surface of the material.

In a specific illustrative embodiment, mammalian xanthine oxidase (XO) is used as the surface catalyst for nitrite reduction to NO. In the presence of

nicotinamide adenine dinucleotide (NADH), or other reducing equivalents in blood, the surface catalyst will generate NO as the nitrite ions leak from within the material into this surface layer via exchange for chloride and bicarbonate within the blood. A schematic representation of this embodiment of the invention is illustrated in Fig. 1. Referring to Fig. 1, a polymer matrix 11 that has been loaded with a lipophilic nitrite/nitrate salt of tridodecylmethylammonium 12 (R^+NO2^-) that provides a source of nitrite ions (NO_2^-). A coating 13 of xanthine oxidase 13 (XO).

Preliminary feasibility studies have been carried out to demonstrate the basic concept of this invention. Using xanthine oxidase as a model enzyme for nitrite reductase activity. PVC polymer films were doped with $TDMA^+$ NO_2^- and then coated with a layer of immobilized XO.

Illustratively, the PVC polymeric film, or membrane, was prepared by a cocktail solution casting method as described, for example, in Mathison, et al., Anal. Chem., Vol. 71, pages 4614-4621 (1999) or any of the patents referenced herein. The cocktail solution was prepared by dissolving the appropriate amounts of membrane components (polymer, plasticizers and, in some cases, an ion-exchanger) into a solvent, illustratively tetrahydrofuran (THF). The membranes were cast in a mold to a final thickness of about 150 μm.

The polymer film was then coated with immobilized XO prepared by crosslinking XO with bovine serum albumin (BSA) in the presence of glutaraldehyde. The cross-linked product forms a hydrogel that is dip-coated on the PVC polymer substrate.

An electrochemical sensor was used to probe the surface concentrations of NO generated when the coated film were placed into a buffered solution containing NADH at physiological pH. Significant levels of NO were generated at the surface of the film under these conditions. The generation of NO near the surface of the polymer film continued for several hours as the nitrite in the film was exchanged for anions in the buffer phase.

In this particular embodiment, the electrochemical NO sensor used was similar in style to conventional Clark type oxygen sensor. Glass coated Platinum (Pt) wire served as the anode and Ag/AgCl wire (0.25 mm dia.) was used as the cathode. The internal filling solution was composed of 0.3 mM HCl and 30 mM NaCl, pH 3.5. An outer gas permeable membrane (Goretex, polytetrafluoroethylene with 50% porosity and 0.2 μm pore size) was placed between the internal filling solution and sample solution. Amperometric NO measurements were performed using an electrochemical analyzer.

Fig. 2 graphically illustrates that, when a similar film coated with XO was exposed to whole sheep blood, without adding any reducing equivalents in the form of NADH, measurable levels of NO were generated at the surface of the film as detected by the aforementioned electrochemical NO sensor. This data suggests that there is adequate endogenous reducing equivalent species in blood to serve as the source of electrons for the biocatalytic reaction at the surface of a polymer prepared in accordance with the present invention.

In another illustrative embodiment, biomimetic catalysts, such as Cu(II)-ligand complexes, for example, dibenzo[e,k]-2,3,8,9-tetraphenyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene, were either incorporated in or tethered to a polymer or other material surface, such as a metal. Examples of this embodiment is shown in Figs. 3 and 4.

Fig. 3 is a schematic representation of a polymer matrix 31, illustratively PVC, that has been loaded with a lipophilic Cu(II) ligand complex 32 as well as a lipophilic nitrite/nitrate salt of tridodecylmethylammonium 33 (N^+NO2^-) that provides a source of nitrite ions (NO_2^-) in the polymer. When the polymer is exposed to an aqueous solution containing ascorbate (ASC) or ascorbic acid, the ascorbic acid reduces the Cu(II) in the ligand complex to Cu(I). The Cu(I) in turn reduces nitrites in the film to NO.

Fig. 4 is a schematic representation of a material 40 that has a catalytic surface 41 created by tethering a Cu(II) ligand complex 42 to the surface. When the catalytic

surface is exposed to an aqueous solution, which may be blood, containing ascorbic acid, the ascorbic acid reduces Cu(II) in the ligand to Cu(I). The Cu(I) returns to Cu(II) thereby converting nitrites and nitrosothiol (RSNO), for example, in the solution to NO.

Fig. 5 is a graphical representation of the surface generation of NO from a Cu(II) ligand complex-containing polymer film in a bulk solution containing nitrite and ascorbate. The data is plotted as NO concentration in parts per billion (ppb) as a function of time in seconds.

Three films having the following formulation were prepared in accordance with the method set forth above: 66.7 wt% PVC polymer (132 mg); 33.3 wt% plasticizer, illustratively nitrophenyloctyl ether (NPOE; 66 mg), and a Cu(II) ligand complex, CuL_xCl2 (2 mg), L_x being ligands, L1-L3 as shown on Fig. 6. The illustrative metal ligand complexes, specifically Cu(II) ligand complexes, shown on Fig. 6 are dibenzo[e,k]-2,3,8,9-tetraphenyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene labeled L1; dibenzo[e,k]-2,3,8,9-tetramethyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene labeled L2; and labeled L3 .

Although these complexes are shown as chloride salts, it is to be understood that other counterions are appropriate. Other metal ions were evaluated for activity, *i.e.*, ability to mediate the reduction of nitrite to NO by ascorbate, including Co(II), Ni(II), Zn(II) Mn(II), Al(II), and Fe(III). Of these ions, only Fe(III) yielded a detectable level of NO, but this was far less than that observe with Cu(II) under identical conditions. Other metals, such as V(III), Cr(III), and Ti(III) have also been suggested as being capable of reducing nitrite to NO. However, unlike Cu(II) (or Fe), these metals are not present in appreciable levels *in vivo*, either within physiological fluids, or within specialized cellular vesicles. Therefore, Cu(II) is presently the preferred metal ion for the practice of the invention.

Referring back to Fig. 5, traces 61, 62, and 63 being ligands L1-L3, respectively. In this particular experiment, the bulk solution was deoxygenated phosphate buffered saline (PBS) having a pH of 7.4. At time t=0, 1 mM nitrite and

1 nM ascorbate were added to the PBS solution and NO generation was measured with a chemiluminescense detector. The results demonstrate that films in accordance with the present invention are capable of NO generation at the interface when the nitrites and ascorbates are in the bulk solution, such as would occur when the films were placed in contact with blood in an *in vivo* situation.

Fig. 7 is a graphical representation of NO generation from a nitrite ion pair/Cu(II) complex, specifically the complex designated L2 on Fig. 5A, doped into a polymer film. The data is plotted as NO concentration in parts per billion (ppb) as a function of time in minutes following introduction of 1mM ascorbate into a deoxygenated PBS solution having pH 7.4.

The polymeric film compositions used in this experiment are as follows:

Film 1:

66 mg PVC; 132 mg NPOE; 4 mg Cu(II) complex; and 20 mg ion pair, or$TDMA^+NO_2^-$

Film 2:

100 mg PVC, 100 mg NPOE, 4 mg Cu(II) complex; and 20 mg ion pair

Film 3:

132 mg PVC; 66 mg NPOE; 4 mg Cu(II) complex; and 20 mg ion pair

These results show generation of NO by the polymer film that is particularly good for the highly plasticized embodiments.

The major advantage of this technology over the previous methods for generating

NO locally at the surface of polymers or other materials is the potential simplicity of simply dip-coating the material with a biocatalytic or biomimetic catalytic layer. The catalytic layer may have a single catalyst or mixture of reductase activities. It can be a biological protein (enzyme) or it can be a metal ion ligand complex that mimics the enzyme function. Even in those embodiments where added $TDMA^+$ NO_2^-/NO_3^- or some other nitrite/nitrate salt, or a nitrosothiol, such as nitroso cysteine, is required,

or desired, within the polymer, the stability of such species is likely to far exceed the stability of diazeniumdiolates and other NO donors used to date.

In a clinical situation, it should be noted that, even if the amount of reducing equivalent species in the blood were to vary from test subject to test subject, it is possible to add reducing equivalents of an alternate electron donor to the blood, illustratively in the form of ascorbic acid, by administering low doses Vitamin C to the patient. This ensures the presence of adequate levels of reducing equivalents.

Although the invention has been described in terms of specific embodiments and applications, persons skilled in the art can, in light of this teaching, generate additional embodiments without exceeding the scope or departing from the spirit of the invention described herein. Accordingly, it is to be understood that the drawing and description in this disclosure are proffered to facilitate comprehension of the invention, and should not be construed to limit the scope thereof.

WHAT IS CLAIMED IS:

1. A material having a catalytic surface that has immobilized, or available at the surface thereof, a catalytic agent having nitrite reductase and/or nitrite reductase-like activity, or nitrosothiol reductase activity, which converts nitrite/nitrate or nitrosothiols to nitric oxide when the catalytic surface is in contact with blood.

2. The material of claim 1 wherein the catalytic agent is a biocatalytic agent.

3. The material of claim 2 wherein the biocatalytic agent is an enzyme having nitrite reductase and/or nitrite reductase-like activity, or a nitrosothiol reductase activity.

4. The material of claim 2 wherein the enzyme is selected from the group of nitrite reductases, nitrate reductases, enzymes having nitrosothiol reducing activity, and xanthine oxidase, or combinations thereof.

5. The material of claim 3 wherein the enzyme is xanthine oxidase.

6. The material of claim 1 wherein the catalytic agent is a biomimetic catalytic agent.

7. The material of claim 6 wherein the biomimetic catalytic agent is a metal ion ligand complex wherein the metal ion is capable of reducing one or more of nitrite, nitrate, nitrosothiols, and other nitrogen-containing blood species to nitric oxide.

8. The material of claim 7 wherein the biomimetic catalytic agent is a Cu(II) metal ion ligand complex.

9. The material of claim 8 wherein the Cu(II) metal ion ligand is selected from the group consisting of dibenzo[e,k]-2,3,8,9-tetraphenyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene; dibenzo[e,k]-2,3,8,9-tetramethyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene; and dibenzo[e,k]-2,3,8,9-tetraethyl-1,4,7,10-tetraaza-cyclododeca-1,3,7,9-tetraene.

10. The material of claim 1 wherein the material is selected from the group consisting of polymers, metals or carbon (graphite).

11. The material of claim 10 wherein the material is a polymer.

12. The material of claim 11 wherein the polymer is selected from the group of poly(vinyl chloride), polyurethane, and silicone rubber.

13. The material of claim 11 wherein the polymer further includes lipophilic salts of nitrite/nitrate or nitrosothiols within the polymer matrix to create a reservoir of nitrite/nitrate or nitrosothiol that can continuously leak to the catalytic surface.

14. The material of claim 13 wherein the lipophilic salt of nitite/nitrate is tridodecylmethylammonium nitrite ($TDMA^+ NO_2^-/NO_3^-$).

15. The material of claim 10 wherein the material is a metal.

16. The material of claim 15 wherein the metal is selected from the group consisting of stainless steel, nickel, titanium, aluminum, copper, gold, silver, platinum and alloys or combinations thereof.

17. The material of claim 15 wherein the catalytic agent is covalently attached to the surface of the metal.

18. The material of claim 15 wherein the surface of the metal is coated with a polymeric film having the catalytic agent incorporated into the matrix or attached to the surface of the polymeric film.

19. The material of claim 18 wherein the polymeric film further includes lipophilic salts of nitrite/nitrate or nitrosothiols within the polymer matrix to create a reservoir of nitrite/nitrate or nitrosothiol that can continuously leak to the catalytic surface.

20. A material comprising:

a hydrophobic polymer substrate; and

a catalytic agent having nitrite reductase and/or nitrite reductase-like activity, or a nitrosothiol reductase activity attached to a surface of the hydrophobic polymer substrate to form a catalytic surface.

21. The material of claim 20 further including, within the polymer substrate, lipophilic salts of nitrite/nitrate or nitrosothiols within the polymer matrix to create a reservoir of nitrite/nitrate or nitrosothiol that can continuously leak to the catalytic surface.

23. The material comprising:

a metal substrate; and

a biomimetic catalytic agent covalently attached to the surface.

24. The material of claim 23 wherein the biomimetic catalytic agent is a metal ion ligand complex wherein the metal ion is capable of reducing one or more of nitrite, nitrate, nitrosothiols, and other blood species to nitric oxide.

25. The material of claim 24 wherein the biomimetic catalytic agent is a Cu(II) metal ion ligand.

26. The material of claim 23 further comprising a polymeric film lipophilic salts of nitrite/nitrate or nitrosothiols within the polymer matrix to create a reservoir of nitrite/nitrate or nitrosothiol that can continuously leak to the catalytic surface.

27. A method of generating NO *in vivo* at the interface of a material surface and blood in response to contact of the surface with blood comprising:

providing catalytic agents at the surface of a material, the catalytic agents having nitrite reductase and/or nitrite reductase-like activity, or a nitrosothiol reductase activity; and

contacting the surface of the material to blood so as to convert nitrite/nitrate or nitrosothiols in the blood to nitric oxide.

28. The method of claim 27 further comprising the step of:

providing a polymeric film on the material that contains a source of lipophilic salts of nitrite/nitrate or nitrosothiols within the polymer matrix to create a reservoir of nitrite/nitrate or nitrosothiol that can continuously leak to the catalytic surface.

29. The method of claim 27 wherein the step of providing a catalytic agent comprises covalently binding or otherwise attaching or making available to the surface, Cu(II) ligand complexes for reducing nitrite/nitrate/nitrosothiols in the blood to NO.

30. The method of claim 27 wherein the step of providing a catalytic agent comprises covalently binding or otherwise attaching or making available to the surface, an enzyme having nitrite reductase and/or nitrite reductase-like activity, or

a nitrosothiol reductase activity, for reducing nitrite/nitrate/nitrosothiols in the blood to NO.

31. A device comprising:

a material having immobilized, or available at a surface thereof, a catalytic agent having nitrite reductase and/or nitrite reductase-like activity, or a nitrosothiol reductase activity, which converts nitrite/nitrate or nitrosothiols to nitric oxide when in contact with blood.

32. The device of claim 32 wherein the the medical device is selected from the group consisting of arterial stents, guide wires, catheters, bone anchors and screws, protective platings, hip and joint implants, spine appliances, electrical leads, biosensors and probes.

Schematic of Surface NO Generation via Nitrite Reductase Activity and Polymer Loaded with Nitrite Salt

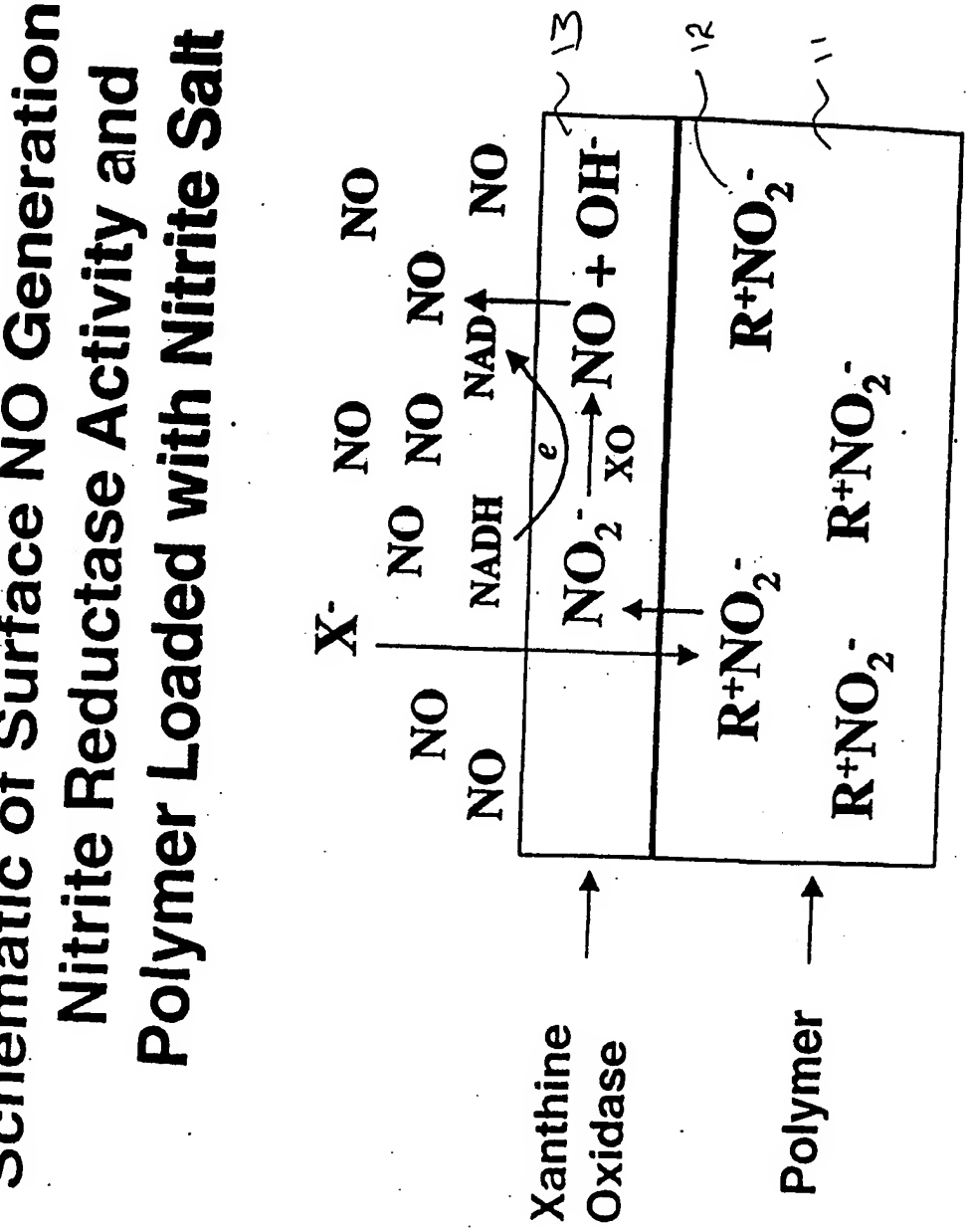
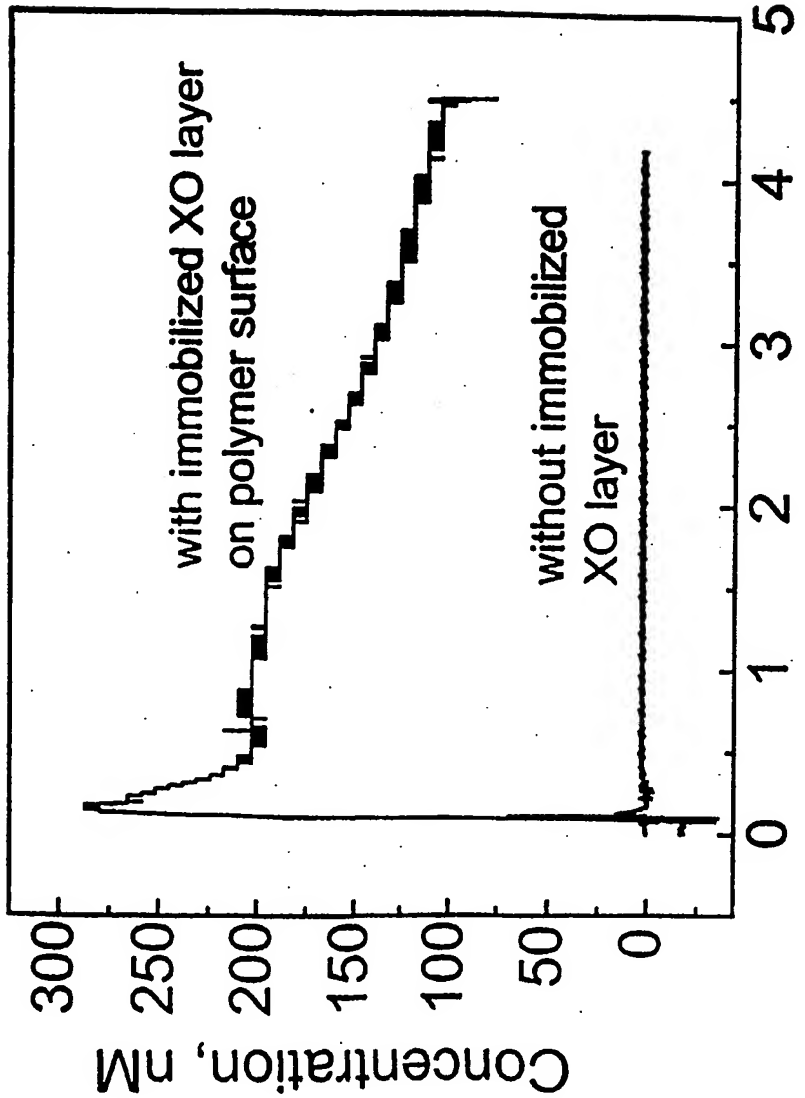


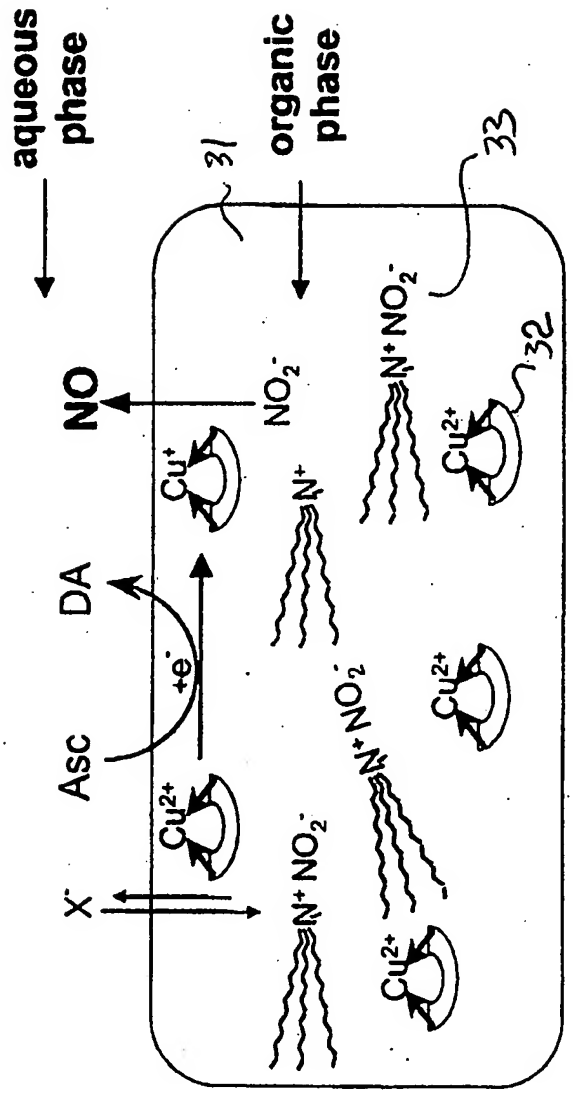
FIG. 1

NO Releasing Profile from Nitrite Ion-Pair Doped Polymer Films in Sheep Blood



*distance between polymer surface and sensor is 10 μm

Electron Transfer from Aqueous to Organic Phase



Asc: Ascorbate, DA: Dehydroascorbate, X⁻: anion

Cu²⁺ : lipophilic copper complex

FIG. 3

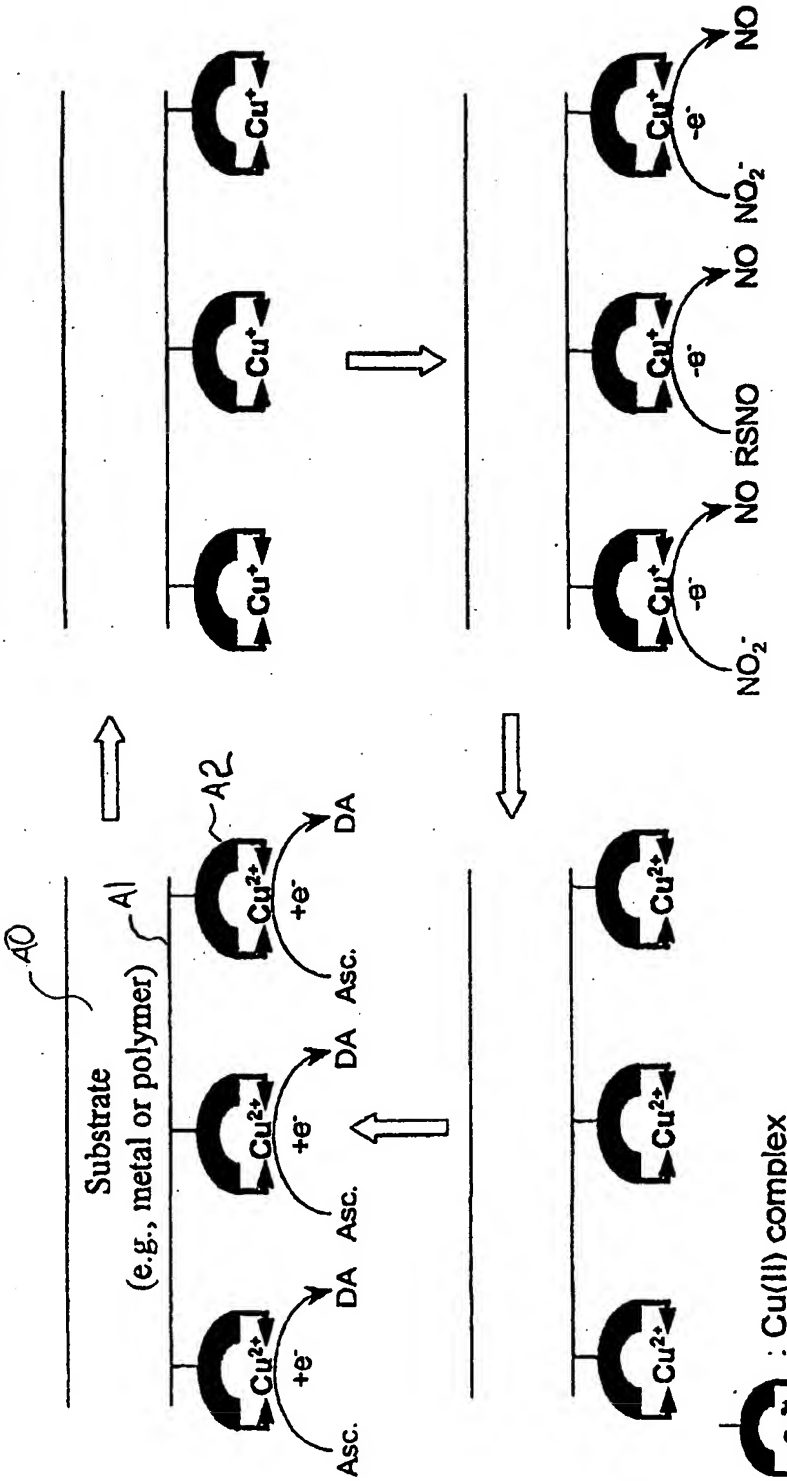
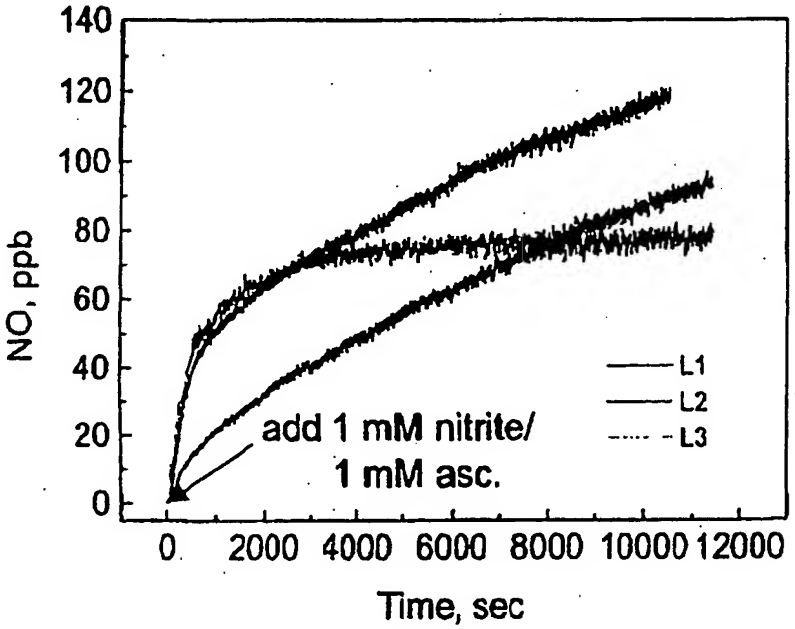

A0
Substrate
(e.g., metal or polymer)
A1
A2
Cu+
Cu2+
+e-
-e-
Asc.
DA
NO
RSNO
NO2-
: Cu(II) complex
Asc. : Ascorbate
DA : Dehydroascorbate
RSNO : nitrosothiol

FIG. 4

NO Generation from PVC Film upon Addition of 1 mM Nitrite/1 mM Ascorbate



Film Formulation:
66.7 wt% PVC (132 mg)
33.3 wt% NPOE (66 mg)
CuL_xCl_2 (2 mg)(x = 1, 2, 3)
cut 1 cm disk from parent film

Background solution : deoxygenated PBS solution, pH 7.4

Fig 5

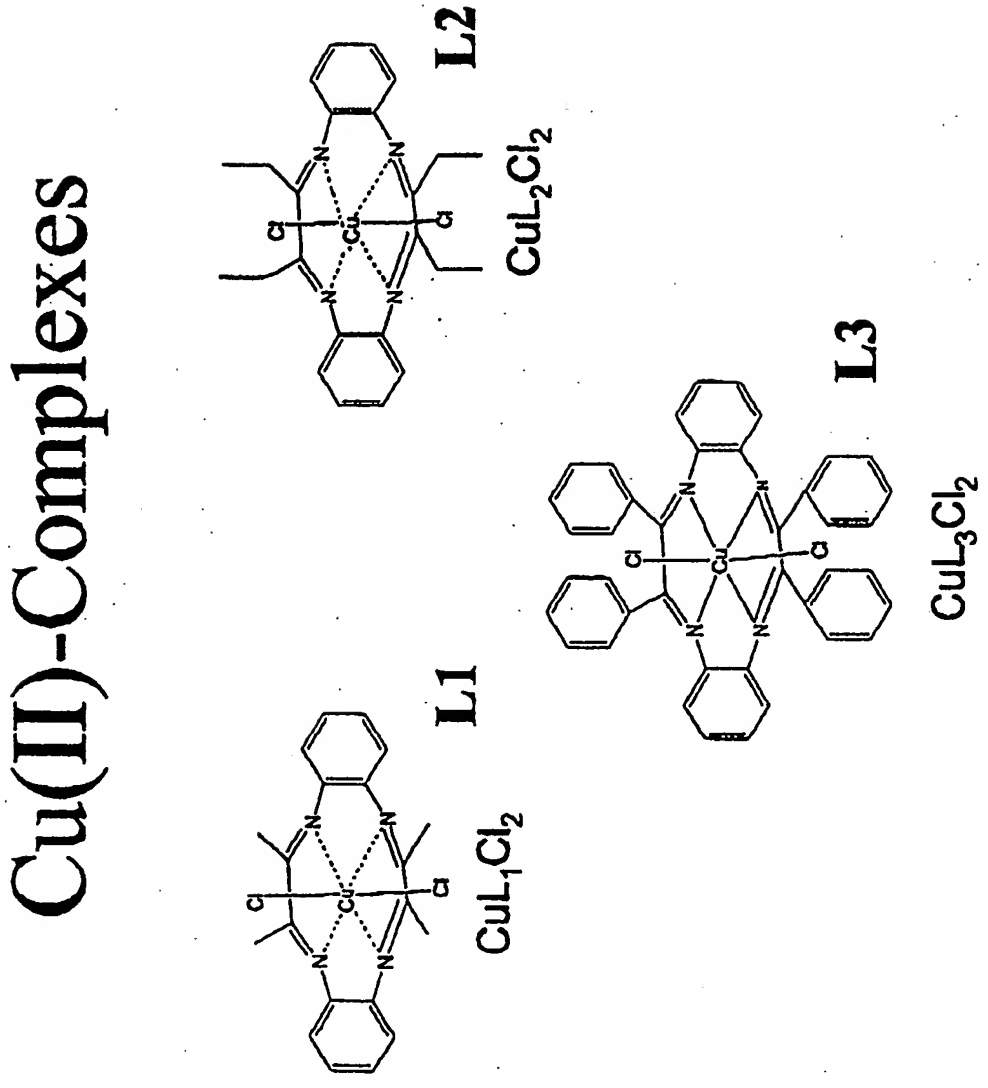
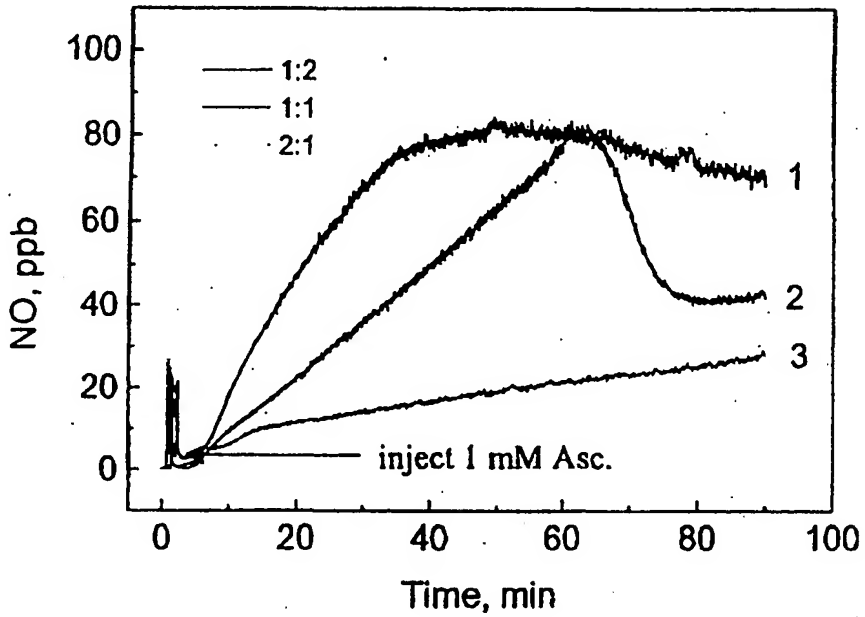


FIG. 1

NO Generation from Nitrite Ion Pair/Cu(II)-complex(L2) Doped PVC Film upon Addition of 1 mM Ascorbate



Film compositions

1 : 66 mg PVC
132 mg NPOE
4 mg Cu(II)-complex
20 mg ion pair

2 : 100 mg PVC
100 mg NPOE
4 mg Cu(II)-complex
20 mg ion-pair

3 : 132 mg PVC
66 mg NPOE
4 mg Cu(II)-complex
20 mg ion-pair

Background solution : deoxygenated PBS, pH 7.4
Each film contains 1.0 mg of $TDMA^{+}NO_2^{-}$

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/01687

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 38/44, 35/34; C12N 11/16, 11/14, 11/02, 11/08

US CL :424/94.4, 630; 435/174, 176, 177, 180

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/94.4, 630; 435/174, 177, 180

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,294,539 A (JOHANNSSEN et al) 15 March 1994, see entire document, especially paragraph bridging columns 4 and 5.	1-21, 23-32
Y	US 6,143,556 A (TRACHTENBERG) 07 November 2000, see entire document, especially column 8, lines 34-35.	1-21, 23-32
Y	US 5,834,030 A (BOLTON) 10 November 1998, see entire document.	1-21, 23-32
Y	US 3,933,589 A (KEYES) 20 January 1976, see entire document, especially column 2, lines 50-51.	1-21, 23-32
Y	US 6,033,368 A (CASTON, IV et al) 07 March 2000, see entire document, especially column 5, lines 38-41.	1-21, 23-32

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
24 MAY 2002	12 JUN 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DAVID M. NAFF
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/01687

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,283,339 A (ARNOLD et al) 01 February 1994, see entire document, especially column 13, lines 50-65 and column 18, lines 44-49.	6-9, 29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/01687

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST
search terms: nitrate reductase, nitrite reductase, immobilized, nitric oxide, nitrosothiol reductase, metal ion ligand complex, Cu(II)